Incorporation of dimethyl sulfoxide to model adhesive resins with different hydrophilicities : Physico/mechanical properties

Al-Ani, Anas Aaqel Salim Salim

2019-05

Al-Ani , A A S S , Stape , T H S , Mutluay , M , Tjäderhane , L & Tezvergil-Mutluay , A 2019 , ' Incorporation of dimethyl sulfoxide to model adhesive resins with different hydrophilicities : Physico/mechanical properties ' , Journal of the Mechanical Behavior of Biomedical Materials , vol. 93 , pp. 143-150 . https://doi.org/10.1016/j.jmbbm.2019.02.015

http://hdl.handle.net/10138/315582
https://doi.org/10.1016/j.jmbbm.2019.02.015

publishedVersion

Journal of the Mechanical Behavior of Biomedical Materials 93 (2019) 143–150

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Incorporation of dimethyl sulfoxide to model adhesive resins with different hydrophilicities: Physico/mechanical properties

Anas Aaqel Salim Salim Al-Ani[a,b,c,*], Thiago Henrique Scarabello Stape[a,b,c], Murat Mutluay[b,c,d], Leo Tjäderhane[e,f,h], Arzu Tezvergil-Mutluay[b,c,g]

[a] Finnish Doctoral Program in Oral Sciences (FINDOS), University of Turku, Institute of Dentistry, Turku, Finland
[b] Department of Cariology and Restorative Dentistry, Institute of Dentistry, University of Turku, Turku, Finland
[c] Adhesive Dentistry Research Group, Biomaterials and Medical Device Research Program, Biocity, Turku, Finland
[d] Department of Prosthetic Dentistry, Institute of Dentistry, University of Eastern Finland, Kuopio, Finland
[e] Department of Oral and Maxillofacial Diseases, University of Helsinki, Helsinki, Finland
[f] Research Unit of Oral Health Sciences, and Medical Research Center Oulu (MRC Oulu), Oulu University Hospital and University of Oulu, Oulu, Finland
[g] Turku University Hospital, TYKS, University of Turku, Turku, Finland
[h] Helsinki University Hospital, Helsinki, Finland

ARTICLE INFO

Keywords:
Dimethyl sulfoxide
Hydrophilic resin
Hydrophobic resin
Physico/mechanical
Water sorption
Microhardness

ABSTRACT

Objective: To understand dimethyl sulfoxide (DMSO) interaction with distinct methacrylate monomer blends and the impact on polymer formation by investigating the combined relationship among degree of resin hydrophilicity, presence of DMSO and specific physico/mechanical properties.

Methods: One hydrophobic (R2) and one hydrophilic (R5) methacrylate-based resins with different monomer compositions were solvated in ascending DMSO concentrations (0, 0.01, 0.1, 1, 5, and 10 w/w %). Neat resins (0 w/w % DMSO) were used as controls. The degree of conversion was determined by Fourier-transform infrared spectroscopy. Polymer crosslinking density was indirectly measured by a modified ethanol-water two-stage solvation technique and the biaxial flexural strength was measured after 24 h and 30 days of water storage at 37 °C. Water sorption and solubility were gravimetrically assisted during 28 days of water storage to determine the kinetics of water-polymer interactions. Data were analyzed by ANOVA and Tukey test ($\alpha = 0.05$).

Results: Incorporation of high DMSO-concentrations significantly increased the degree of conversion of all tested formulations, specifically for the hydrophobic resin ($p < 0.05$). Despite the increase in degree of monomer conversion, higher water sorption/solubility values and lower biaxial flexure strengths were detected as a result of reductions in polymer crosslink density ($p < 0.05$). In general, low DMSO-concentrations had no impact on the biaxial flexural strength, crosslinking density and water sorption/solubility ($p < 0.05$).

Conclusion: DMSO-monomer ratio and monomer composition are critical for new dental methacrylate-based adhesive formulations. High DMSO incorporation hampers physico/mechanical properties of methacrylate bonding resins, albeit to a lesser extend when hydrophilic resins are employed. Nonetheless, DMSO-solvated hydrophobic adhesives extensively outperform their hydrophilic correspondents. DMSO incorporation of 1w/w % may constitute a secure threshold regardless of monomer composition.

1. Introduction

Despite substantial advances in resin-dentin bonding over the past decades, reduction in bonding effectiveness of currently available dental adhesives remains a major limitation in modern adhesive dentistry (Tjäderhane et al., 2013; Pashley et al., 2011). Hydrolysis of both organic and resin constituents of hybrid layer (Frassetto et al., 2016) persists as impediments in dentin bonding longevity. Since bonding is directly related to the quality of the formed polymer (Ferracane, 2006; Bae et al., 2005), the resin components play an important role in proper resin-dentin interaction and in the mechanical properties of the material (Van Landuyt et al., 2007).

Current dental adhesives are essentially co-monomer blends with different hydrophilicity levels solvated in volatile organic solvents to

* Correspondence to: Department of Cariology and Restorative Dentistry, Adhesive Dentistry Research Group, University of Turku, Lemminkaisenkatu 2, FI-20520 Turku, Finland.
E-mail address: aassal@utu.fi (A.A.S. Salim Al-Ani).

https://doi.org/10.1016/j.jmbbm.2019.02.015
Received 15 August 2018; Received in revised form 21 December 2018; Accepted 13 February 2019
Available online 14 February 2019
1751-6161/

enhance mixing and to avoid phase separation (Van Landuyt et al., 2007). Solvents are crucial to facilitate the displacement of water from dentin (Jacobsen and Söderholm, 1998) and to ensure better wetting and monomer penetration into the etched-dentin matrix (Pashley et al., 2007). As hydrophobic crosslinking monomers are necessary to improve the mechanical properties of the polymer network, but are incapable to properly bond to the inherently hydrated dentin (Fang et al., 2012; MRDO et al., 2005), hydrophilic monomers must be included in adhesive formulations for adequate resin-dentin coupling. Unfortunately, increased resin hydrophilicity has been strongly associated with reduced bonding performance over time partially due to higher monomer elution from the polymerized resin matrix and subsequently increased water sorption (Ito et al., 2005; Tay and Pashley, 2003). The presence of residual solvents also produces localized areas of incomplete monomer polymerization throughout the resin matrix (Yiu et al., 2005). In addition, high concentrations of hydrophilic monomers alter the colligative properties of the entire resin mixture (Pashley et al., 1998). Thus, the use of increasing ratios of hydrophilic monomers raises concerns on whether bonding resins have become too hydrophilic in nature (Tay and Pashley, 2003). Since water uptake is dependent not only on the presence of residual solvents but also influenced by the degree of monomer hydrophilicity, the intricate combination of different monomers and solvents, as well as their ratios, also dictate the reliability of bonding resins to sustain long-term degradation in the oral cavity (Pashley et al., 1995).

One successful approach to improve the long-term bonding performance of relatively hydrophilic resins is partial substitution of commonly used organic solvents by dimethyl sulfoxide (DMSO; $(CH_3)_2SO$) (Stape et al., 2016). DMSO is a polar aprotic solvent that dissolves both polar and non-polar compounds. It is a poly-functional molecule, with a highly polar S=O group and two hydrophobic methyl groups, fully miscible in most solvents and in hydrophilic and hydrophobic monomers commonly used in adhesive dentistry. Monomer conversion can be technically improved by DMSO incorporation in poly-methacrylate resin blends due to lowering of termination rates in free radical polymerization (Gupta and Nandi, 1970) and by disruption of monomer intermolecular attraction more efficiently than commonly used solvents. However, the benefits reported for HEMA-containing hydrophilic resin blends are concentration-dependent: higher DMSO concentrations impaired the mechanical properties of resins containing relatively high HEMA/BisGMA ratios (Stape et al., 2016). In such DMSO-solvated HEMA-rich resin blends, the lower availability of crosslinking monomers (*i.e.* BisGMA) combined with the reduced approximation between growing polymer chains may synergistically compromise polymer crosslinking and thus hamper polymer mechanical properties. Although the impact of DMSO on relatively hydrophilic ethanol-solvated resins has been reported (Stape et al., 2016), there is no information regarding the effect of DMSO on resin blends with different monomer composition containing higher ratios of crosslinking monomers. Hitherto, there are no studies where the relationship among the presence of DMSO, degree of resin hydrophilicity and physico/mechanical properties of commonly used BisGMA-TEGDMA-HEMA bonding resins have been investigated together to better understand the effect DMSO on polymer network formation and durability.

Therefore, the aim of this study was to evaluate the DMSO solvation effect of a hydrophobic resin with high fractions of crosslinking monomers and a hydrophilic resin blend containing low BisGMA/HEMA ratios in ascending DMSO (0.01, 0.1, 1, 5 and 10 w/w %) aliquots. We measured the degree of conversion after polymerization, polymer crosslink density and biaxial flexural strength at 24 h and after 30 days of water storage, as well as water sorption and solubility. The null hypotheses were as follows: there would be no effect of DMSO incorporation on hydrophobic or hydrophilic resins in terms of (*i*) degree of conversion; (*ii*) polymer crosslink density; (*iii*) mechanical properties; and (*iv*) water sorption and water solubility.

Table 1
Composition of the experimental bonding resins solvated in DMSO.

	Resin	Composition	% (w/w%)
Neat resin	R2	BisGMA	70.00
Hydrophobic resin		TEGDMA	28.75
Batch# 727–206–2		CQ	0.25
		EDMAB	1.00
Neat resin	R5	BisGMA	40.00
Hydrophilic resin		HEMA	28.75
Batch# 727–206–5		2MP	30.00
		CQ	0.25
		EDMAB	1.00
Solvated resins	R2 or R5 + 0.01% DMSO	Neat resin	99.99
		DMSO	0.01
	R2 or R5 + 0.1% DMSO	Neat resin	99.90
		DMSO	0.1
	R2 or R5 + 1% DMSO	Neat resin	99.00
		DMSO	1
	R2 or R5 + 5% DMSO	Neat resin	95.00
		DMSO	5
	R2 or R5 + 10% DMSO	Neat resin	90.00
		DMSO	10

Abbreviations: BisGMA = bisphenol A diglycidyl ether dimethacrylate; TEGDMA = triethylene-glycol dimethacrylate; CQ = camphorquinone; EDMAB = ethyl N, N-dimethyl-4-aminobenzoate; HEMA = 2-hydroxyethyl methacrylate; 2MP = Bis [2-(methacryloyloxy) ethyl] phosphate; DMSO = dimethyl sulfoxide.

2. Materials and methods

2.1. Specimen preparation

Two neat methacrylate-based experimental resins (R2 and R5, Bisco Dental Product Co, Schaumburg, IL, USA) of known compositions and hydrophilicities (Table 1) were used to prepare twelve experimental bonding resins containing ascending DMSO concentrations. The hydrophobic (R2) and hydrophilic (R5) resins were solvated in DMSO to produce bonding resins with final DMSO concentration of 0, 0.01, 0.1, 1, 5 and 10 w/w % and DMSO-resin blends were magnetic stirred (VWR international, Lutterworth, UK) to ensure homogenous mixtures. Neat resins were used as controls. Disc shaped specimens (0.5 ± 0.02 mm in thickness and 6 ± 0.1 mm in diameter) were prepared using custom-made stainless steel molds. A Mylar strip was placed on the top of a glass slide followed by the mold. To produce samples with smooth-flat surfaces and to eliminate the oxygen inhibition layer, 25 μL of each experimental resin was placed inside the mold followed by a Mylar strip and a glass slide. Eventual air bubbles were carefully removed using a micro-brush and subsequently both glass slides were clamped together to produce discs with fixed thickness. A light-emitting diode (LED) light curing unit (Elipar, 3M ESPE, Seefeld, Germany) with an output of 600 mW/cm^2 was used to light-cure the adhesive discs for 20 s on both sides with the tip at 1 mm distance from the specimen surface. After curing, specimens were dry- or wet-stored in distilled water at 37 °C in darkness for 24 h, depending on the test method and sample dimensions were measured to the nearest 0.01 mm using a digital micrometer (Metteler Toledo, Columbus, OH, USA).

2.2. Degree of conversion

Absorption spectra of uncured and cured experimental adhesives were obtained by Fourier Transform Infrared Spectroscopy (FTIR; Spectrum one, Perkin Elmer, Beacons filed, Bucks, UK) equipped with a universal attenuated total reflectance (ATR) accessory using a baseline method (Rueggeberg et al., 1990). Monomer conversion was determined by measuring the decrease of the C=C ratio before and after polymerization to an internal aromatic C=C standard. A circumferential silicon hollow mold (0.6 mm thickness, 6 mm diameter) was centralized over the ATR crystal surface and a 5-μL drop of each experimental

adhesive (n = 5/group) was placed inside the mold in direct contact with the ATR crystal. A Mylar strip was immediately placed over the top of resin to exclude oxygen and prevent solvent evaporation. Photoactivation was performed at a fixed 1 mm tip distance. Infrared spectra collected before and after polymerization with a LED light-curing unit (Elipar, 3M ESPE) for 20 s. The light intensity was checked regularly. After light curing, post-cure polymerization was allowed to continue up to 300 s and the absorption spectrum was collected for each sample. Degree of conversion (DC) was calculated by changes in C=C absorption peak ratios of aliphatic (1638 cm^{-1}) and aromatic (1608 cm^{-1}) peaks in both uncured and cured states obtained from the infrared spectra according to the following equation:

$$DC(\%) = \left(\left(1 - \frac{R^{(Cured)}}{R^{(Uncured)}}\right)\right)X \quad 100$$

Where "R" is the ratio of aliphatic and aromatic peak intensities at 1638 cm^{-1} and 1608 cm^{-1} in cured and uncured adhesives.

2.3. Softening in ethanol

Polymer crosslink density was indirectly measured by a modified ethanol-water two-stage solvation technique adapted from standard softening tests (Moraes et al., 2007; Schneider et al., 2008; Leitune et al., 2013). Circular samples (n = 7/group) were fabricated in stainless steel mold and dry-stored at 37 °C in the dark for 24 h. Samples were subsequently cold-embedded in epoxy resin (Struers, Ballerup, Denmark) and highly polished (MetaServ 250 Grinder-Polish, Buehler Ltd, Lake Bluff, IL, USA) with a felt disc saturated with alumina. The initial Knoop microhardness number (KHN1) was registered from three indentations (10 g/ 5 s) 100 μm apart from each other in each specimen using a digital hardness Knoop tester (HMV 2, Shimadzu Corp., Tokyo, Japan) and averaged for statistical purposes. The samples were then immersed in distilled water at 37 °C for 24 h (KHN2) and subjected to softening in absolute ethanol at 37 °C for 4 h (KHN3). After both immersion periods, Knoop microhardness was measured and the percentage reductions were calculated (ΔKNH%) to provide an estimation of polymer crosslink density.

2.4. Biaxial flexural test

Resin discs (n = 6/group) were tested after 24 h and 30-day storage in distilled water at 37° C. Specimens were placed in a custom-made jig and tested in biaxial flexural on a testing machine (Shimadzu, Shimadzu Corp., Kyoto, Japan) at 1 mm/min until fracture maximum loads (N) were recorded for each specimen. The following three equations were used to calculate the biaxial flexural strength:

$$\sigma = -0.2387P(X-Y)/d2$$

$$X = (1+\nu)In(r_2/r_3)^2 + [(1-\nu)/2](r_2/r_3)^2$$

$$Y = (1+\nu)[1+In(r_1/r_3)^2] + (1-\nu)(r_1/r_3)^2$$

Where σ is the biaxial flexural strength (MPa); P is the maximum load causing fracture (N); d is the thickness of the specimen (mm); ν is the Poisson's ratio (used ν = 0.25); r_1 is the radius of the support circle (mm); r_2 is the radius of the loaded area (mm); and r_3 is the radius of the specimen (mm). *In* corresponds to natural logarithm.

2.5. Water sorption (W_{sp}) and solubility (W_{su})

Water sorption and solubility were determined according to ISO 4049 recommendations except for specimen dimensions (0.5 ± 0.02 mm in thickness and 6 ± 0.1 mm in diameter) and storage period, which was extended to 28 days. Specimens (n = 7/group) were placed in desiccator containing dry silica at 37 °C and daily weighed in a calibrated digital balance with 0.01 mg resolution until a constant weight (M1) for each specimen was obtained. Constant weight was considered when mass variation was less than 0.1 mg in a 24 h period. Specimens were then individually placed in sealed plastic vials containing 5 ml of distilled water and stored at 37 °C for different time intervals (1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days). After each time interval, specimens were rinsed in distilled water and gently blot dried to remove any visible water, weighted and immediately returned back to the plastic vials after replacing the distilled water. The initial mass determined after the first desiccation process (M1) was used to calculate mass changes after each fixed time interval during 28 days of water storage. After the 28th day of storage (M2), specimens were dried once again in the desiccator until a constant mass (M3) was reached. Water sorption (W_{Sp}) and water solubility (W_{Su}) after 28 days were calculated (μg/mm^3) using the following equations:

$$Wsp = \frac{(M2 - M3)}{V} Wsu = \frac{(M1 - M3)}{V}$$

Where *M1* is the constant initial mass (μg) of the specimen; *M2* is the mass (μg) of the specimen after immersion in water; *M3* is the constant mass (μg) of specimen after the second desiccation process until constant mass was obtained; *V* is the volume (mm^3) of a specimen.

2.6. Statistical analysis

After confirming data normality (Shapiro-Wilk test) and homoscedasticity (Levene test), biaxial flexural strength, degree of conversion and ΔKNH% data were evaluated separately using two-way ANOVA. Water sorption, water solubility and Knoop microhardness data were evaluated separately by three-way ANOVA. *Post hoc* analyses were performed with Tukey test (α = 0.05). SPSS Statistics, version 23, was used for statistical calculations (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Degree of conversion

Degree of conversion means and standard deviations are shown in Fig. 1. Two-way ANOVA revealed that "Resin type" ($p < 0.001$), "DMSO concentration" ($p < 0.001$) and their interaction ($p < 0.001$) significantly affected degree of conversion. R5 produced significantly higher conversions than R2 for all DMSO concentrations. DMSO incorporation up to 1% produced no effects on the degree of conversion of R2, while incorporation of 5% and 10% DMSO produced significantly higher monomer conversions than the control group in the order of 12% and 22%, respectively. A similar trend was observed for R5 with significant increases of 4% and 14% for resins containing 5% and 10%, respectively.

3.2. Softening in ethanol

Microhardness and ΔKNH% reduction means and standard deviations for all groups are shown in Fig. 2. Three-way ANOVA revealed that "DMSO concentration" ($p < 0.001$) and "resin type" ($p < 0.001$), "storage condition" ($p < 0.001$) and their interaction ($p < 0.001$) significantly affected bonding resins' microhardness. In general, R2 resins presented significantly higher microhardness values compared to R5 resins considering the corresponding storage conditions. DMSO incorporation up to 1% produced no significant changes in the microhardness values of R2 resins after dry-storage, while 5% and 10% significantly reduced the microhardness values in the order of 33% and 45%, respectively. Similar reduction was observed after water and ethanol storage. Water-storage produced no significant changes for R2 resins regardless of DMSO incorporation when comparing to corresponding dry resins. Storage in ethanol presented significant 55–70% reductions in microhardness values compared to dry- and water-storage.

R5 resins also presented significant reductions in microhardness

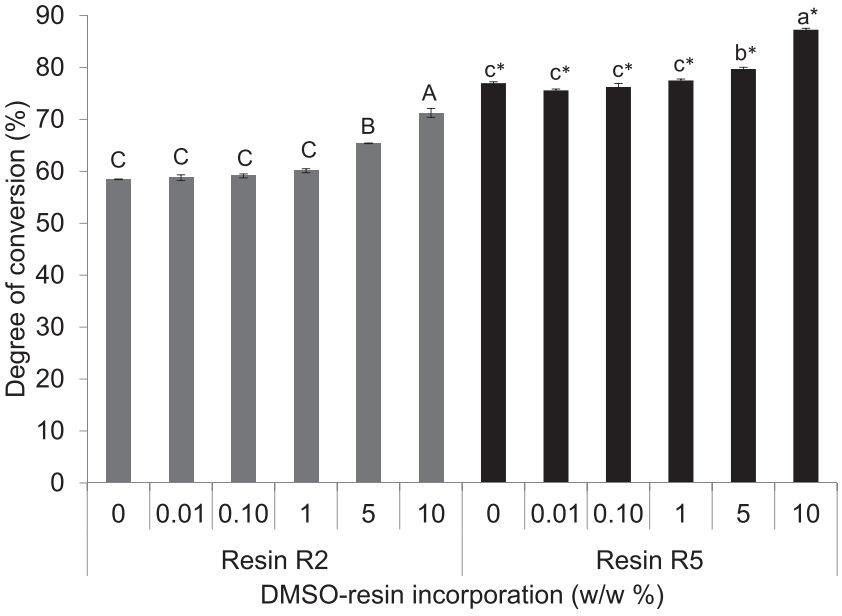

Fig. 1. Degree of conversion means and standard deviations (n = 5) of model hydrophobic (R2) and hydrophilic (R5) resins solvated in ascending DMSO concentrations. Different capital letters indicate significant differences between DMSO concentrations for R2 according to Tukey test ($p<0.05$). Different lowercase letters indicate significant differences between DMSO concentrations for R5 according to Tukey test ($p<0.05$). * indicates significantly higher conversion degrees considering the corresponding DMSO concentration between R2 and R5 according to Tukey test ($p < 0.05$).

after 5% and 10% DMSO incorporation, which was consistent for all storage conditions. Unlike R2 resins, significant reductions of approximately 70% occurred after water-storage when compared to the corresponding control dry samples. Further significant reductions also occurred after ethanol-storage. Two-way ANOVA revealed that "resin type" ($p < 0.001$), "DMSO concentration" ($p < 0.001$) and their interaction ($p < 0.001$) significantly affect ΔKNH% reductions. In general, R5 resins presented significantly higher ΔKNH% reductions (77–88%) compared to R2 resins (56–70%). Both resins presented a similar pattern where 5% and 10% DMSO incorporation produced

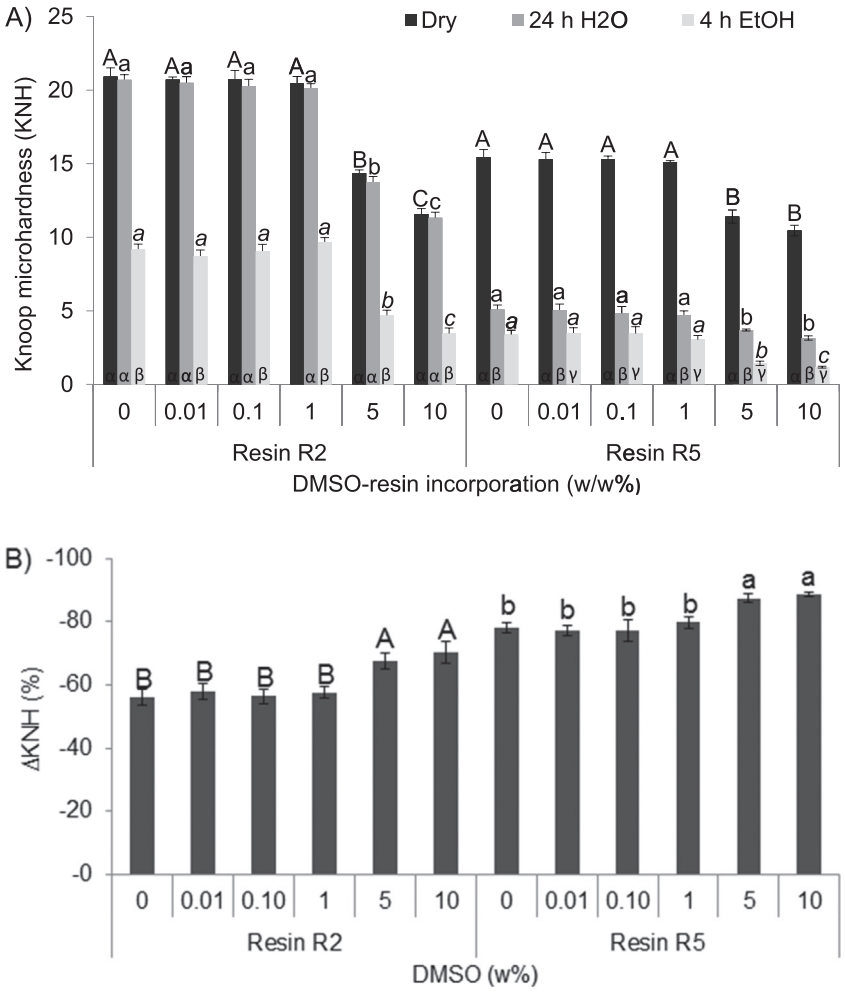

Fig. 2. (A) Microhardness and (B) ΔKNH% reduction means and standard deviations (n = 6) of model hydrophobic (R2) and hydrophilic (R5) resins solvated in ascending DMSO concentrations after immersion in distilled water for 24 h and in absolute ethanol for 4 h at 37 °C. In Figure A, for each resin type (*i.e.* R2 or R5) different capital letters indicate significant differences between DMSO concentrations after dry storage, and different lowercase letters indicate significant differences between DMSO concentrations after water storage. Different italic lowercase letters indicate significant differences between DMSO concentrations after ethanol-storage; and different Greek letters indicate significant differences between storage conditions for each DMSO concentration according to Tukey test ($p<0.05$). For ΔKNH% (Figure B), different capital letters indicate significant differences between R2 resins and different lowercase letters indicate significant differences between R5 resins.

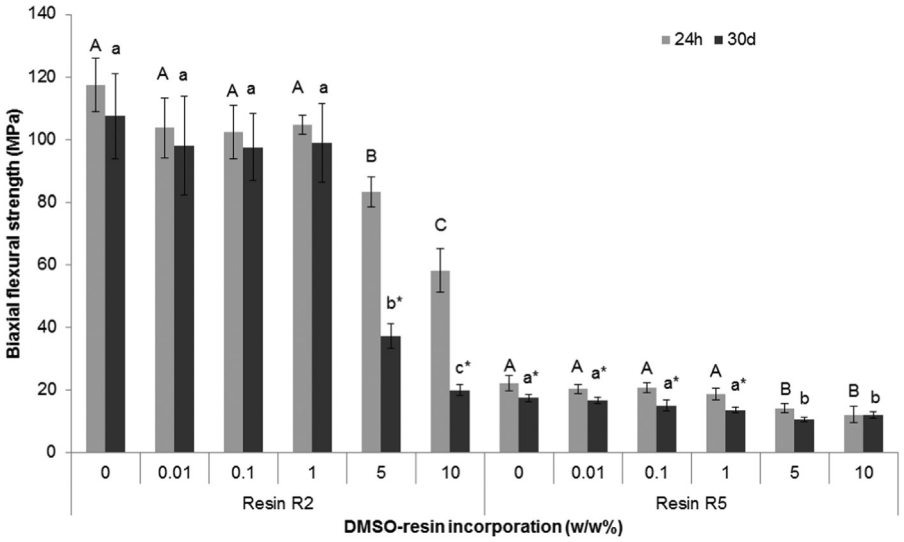

Fig. 3. Biaxial flexural strength means and standard deviations (n = 6) of model hydrophobic (R2) and hydrophilic (R5) resins solvated in ascending DMSO concentrations tested after 24 h and 30 days of water storage at 37°C. R2 and R5 resins were separately analyzed. Different capital letters indicate significant differences between DMSO concentrations at 24 h and different lowercase letters indicate significant differences between DMSO concentrations at 30 days (Tukey test, $p<0.05$). * indicates significant differences between specific concentrations at different incubation times (Tukey test, $p<0.05$).

significantly higher ΔKNH% reductions.

3.3. Biaxial flexural test

Flexural strength means and standard deviations of hydrophobic (R2) and hydrophilic (R5) resins solvated in ascending DMSO concentrations are shown in Fig. 3. Two-way ANOVA revealed that "DMSO concentration" ($p < 0.001$), "storage time" ($p < 0.001$) and their interaction ($p < 0.001$) significantly affected the flexural strength of resin R2 and R5. R2 resins produced higher flexural strength values compared to resin R5 resins. Incorporation of DMSO up to 1% in R2 produced no significant changes at 24 h; however, incorporation of 5% and 10% DMSO produced significant reductions of roughly 30% and 50% compared to control group, respectively. For the samples stored for 30 days in water, significant reductions in flexural strength were observed after 5% and 10% DMSO incorporation of roughly 65% and 80% compared to the control group, respectively. DMSO incorporation up to 1% did not affect the flexural strength of R5, while 5% and 10% DMSO caused significant reductions at 24 h. Similarly, R5 samples stored for 30 days also presented significant reductions after 5% and 10% DMSO incorporation of roughly 40% and 30% compared to control group, respectively, but to a lesser extent when compared to R2 resins.

3.4. Water sorption and solubility

Water sorption and solubility means and standard deviations shown in Fig. 4. Two-way ANOVA revealed that "DMSO concentration" ($p < 0.001$) and "resin type" ($p < 0.001$) and their interaction ($p < 0.001$) significantly affected water sorption. "DMSO concentration" ($p < 0.001$) and "resin type" ($p < 0.001$) significantly affected water solubility. DMSO incorporation in R2 up to 1% produced no changes in water sorption and solubility levels compared to the control group. R2 containing 5% and 10% DMSO presented a significant 1.5- and 2-fold increase in water sorption and 5.3- and 16.5-fold increase in water solubility levels compared to the neat control resin, respectively. R5 presented significantly higher sorption and solubility levels compared to resin R2 irrespective of DMSO concentrations. Significant increases in R5 sorption and solubility levels also occurred at 5% DMSO or above; however, to a lesser extent compared to R2. The exception was R5 containing 10% DMSO, which presented a significant increase of 60% in solubility.

Mass variation curves shown in Fig. 5. Changes in mass percentages were plotted against the storage time in order to obtain the kinetics of water uptake by DMSO-solvated resins during 28 days. All materials showed the greatest mass increase within the first 24 h or 48 h of water storage. DMSO incorporation up to 1% in R2 did not affect water uptake kinetics compared to the neat resin and a negligible decrease in mass occurred after 48 h. In general, higher DMSO incorporation in R2 produced higher maximum mass values. Differently from lower DMSO concentrations, R2 groups containing 5% and 10% DMSO presented a steep decrease in mass variation once maximum values were reached. R5 resins presented no equilibrium after maximum mass values were reached and gradual decreases in mass occurred for all DMSO concentrations, including the control group. Unlike R2, higher DMSO incorporation produced lower maximum mass values, but sharper drops in mass variations.

4. Discussion

The colligative properties of methacrylate-based adhesives are strongly dependent on specific interactions between monomers and solvents (Pashley et al., 1995; Cadenaro et al., 2009). The negative impact of high DMSO incorporation into relatively hydrophilic resins has been previously reported (Stape et al., 2016) and it seems to follow a similar trend as in commonly used volatile solvents (*i.e* ethanol) (Pashley et al., 1995). Nonetheless, current dental adhesives contain both hydrophobic and hydrophobic monomers combined into simplified or multi-mode systems (Van Landuyt et al., 2007). In order to optimize the long term resin-dentin bonding improvement produced by DMSO incorporation into bonding resins (Stape et al., 2016), a deeper understanding of its impact on polymer structure formation of different monomers is of paramount importance. By testing model adhesives with varying hydrophilicities and monomer compositions, our expectations were that hydrophobic resin blends with higher fractions of crosslinking monomers (*i.e.* 70% BisGMA in neat R2 *vs.* 40% in neat R5) would benefit from the increase in monomer conversion produced by DMSO (Stape et al., 2016), even at relatively low concentrations. Indeed, a significant increase in conversion degree occurred, but only with 5% and 10% DMSO incorporation regardless of resin blend composition. Therefore, the first null hypothesis was rejected for DMSO incorporation increased monomer conversion in a concentration-dependent manner.

BisGMA contains a rigid aromatic structure and engages in strong intermolecular hydrogen bonding interactions (Floyd and Dickens, 2006) between the hydroxyl groups (OH) and the carbonyl groups (C=O) in adjacent BisGMA monomers (Lemon et al., 2007). These strong intermolecular interactions in neat and low-DMSO resins compromise monomer mobility during polymerization (Sideridou et al., 2002), resulting in lower conversion. DMSO has two nonpolar methyl groups (CH_3) that may interact with the hydrophobic moieties of BisGMA and

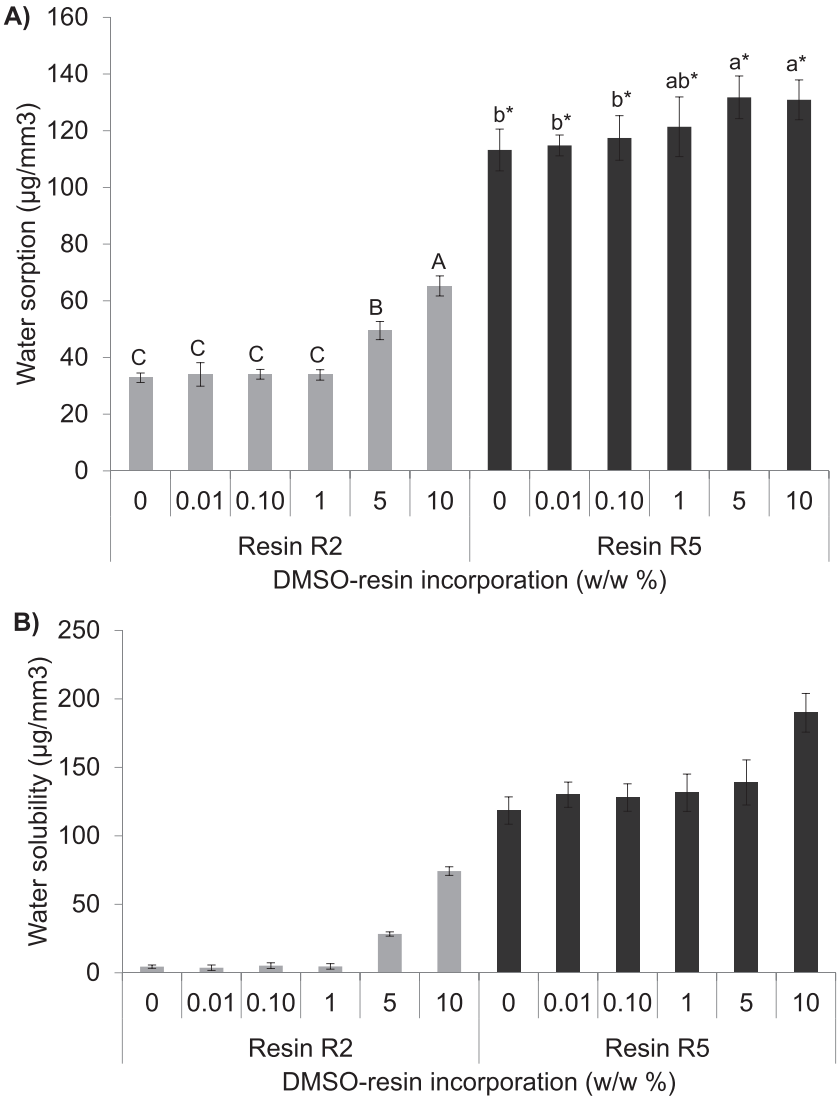

Fig. 4. Water sorption (A) and solubility (B) means and standard deviations (n = 7) of model hydrophobic (R2) and hydrophilic (R5) resins solvated in ascending DMSO concentrations after 28 days of water storage at 37 °C. Different capital letters indicate significant differences between DMSO concentrations for resin R2, different lowercase letters indicate significant differences between DMSO concentrations for R5 (Tukey test, $p < 0.05$). * indicates significant differences between resin R5 and resin R2 for each DMSO concentration.

disrupt monomer intermolecular H-bonding, thus reducing resin viscosity with 5% or higher concentrations. This allows higher mobility of monomer reactive components during curing improving monomer conversion (Cadenaro et al., 2009; Dickens et al., 2003; Holmes et al., 2007). In addition, DMSO also lowers termination rates in methacrylate free radical polymerization (Gupta and Nandi, 1970), which may benefit the degree of conversion.

Nevertheless, higher degree of conversion does not necessarily imply improved polymer structure quality (Ye et al., 2007). In fact, monomer crosslinking has a fundamental role on polymer properties (Ye et al., 2007). Highly crosslinked polymers are more resistant to degradation and solvent uptake, whereas linear polymers contain more pathways for solvent molecules to diffuse within their structure (Ferracane, 2006). For this reason, softening tests are used for indirect assessment of crosslink density (Schneider et al., 2008; Rodrigues et al., 2015). When a polymer comes in contact with polar solvents, hydrogen bonds are formed between the solvent and the polar groups present in the polymer structure (*e.g.* OH, CO). Linear polymer structures are more susceptible for such H-bonding formation because the forces of attraction between low crosslinked polymer chains can be easily exceeded by the forces of attraction between solvents and components of the chains (Ferracane, 2006). Inevitably, disruption of polymer network interchain interactions, alteration of molecular structure and increase in polymer segmental mobility occur depending on the extension of solvent uptake (Ye et al., 2007). As a consequence, ΔKNH% reductions vary depending on the extension of solvent uptake, which can also be influenced by solvent type (Schneider et al., 2008).

In most studies, one standard ethanol or water solution is usually used for softening tests. Here, water and ethanol were sequentially intercalated in a two-step softening protocol to better understand the impact of water on DMSO-incorporate polymer networks. While microhardness values of the experimental DMSO-solvated R2 resins were not affected by water immersion, R5 resins were greatly softened by water likely due to lower fractions of crosslinking monomer BisGMA and overall higher water affinity of the linearly-chain-forming 2-hydroxyethyl methacrylate (HEMA) (Malacarne et al., 2006). Subsequently, ethanol immersion greatly reduced R2 resins microhardness values, but R5 to a lesser extent. This can be explained by the different solubility parameters of the softening solvents and different resin matrix compositions (Ferracane, 2006) of R2 and R5, which clearly produced distinct interactions with water and ethanol. By analyzing ΔKNH % reductions, it is evident that in general R5 resins presented more

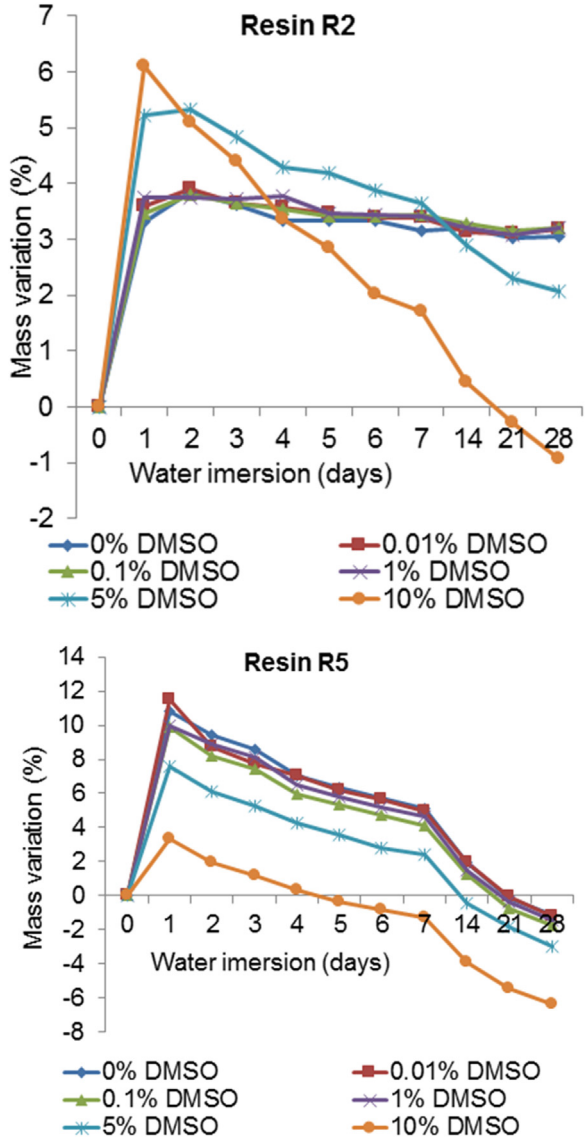

Fig. 5. Mass variation (%) over time of model hydrophobic (R2) and hydrophilic (R5) resins solvated in ascending DMSO concentrations during 28 days of water storage at 37° C. Symbols represent mean values (n = 7).

linear polymeric chains than the higher cross-linked R2 resins. Curiously, DMSO solvation had similar effects on the crosslink density of the tested resins. While DMSO incorporation up to 1% had no substantial effects on crosslink density, 5% and 10% significantly reduced polymer crosslinking, resulting in the rejection of the second null hypothesis. Higher mass percentages of DMSO most likely prevented the approximation between growing polymer chains during curing, leading to reduction of polymer crosslinking (Ye et al., 2007).

Crosslink density results go hand-in-hand with the flexural strength data, especially considering that crosslink density is an important factor to the resultant mechanical properties of polymers (Sideridou et al., 2003). Polymers containing higher ratios of crosslinking monomers (*i.e.* BisGMA and TEGDMA) in general present higher mechanical strengths than more linear polymers (Barszczewska-Rybarek and Jurczyk, 2015), which explains the overall higher biaxial flexure strengths of R2 resins composed of 70% BisGMA and 28.75% TEGDMA (58–117 MPa) compared to R5 resins containing only 40% BisGMA (12–22 MPa). Since relatively low DMSO concentrations were used in some groups, biaxial flexure was selected over conventional uniflexural tests (*i.e.* conventional three or four point bending tests) due to its increased sensitivity and lower data scattering (Pick et al., 2010). Since DMSO incorporation affected the mechanical properties of both tested resins, the third null hypothesis had to be rejected. While lower DMSO amounts had no impact on the mechanical properties, incorporation of 5% and 10% DMSO significantly reduced immediate flexural strengths. The formation of more linear polymeric chains with higher DMSO incorporations contributed to lower mechanical strengths, which is in accordance with previous studies (Sideridou et al., 2003). It is important to note that H-bonding between adjacent linear chains or cross-linked polymerized networks reinforces the three-dimensional polymeric structure (Lemon et al., 2007). It is plausible to assume that incorporation of DMSO in resins over 5% (w/w %) also disrupted polymer interchain H-bonding, thus contributing to lower flexural strengths.

When water penetrates polymer networks, the efficiency of secondary molecular interactions (*i.e.* H-bonding) is reduced, leading to increased polymer plasticization and higher degradation rates (Ferracane, 2006; Ito et al., 2005). Therefore, water storage negatively affected flexural strengths depending on resin type and DMSO incorporation. The flexural strength of R2 resins with low DMSO concentrations was not affected by 30-day water storage; however, significant reductions were observed for resins containing 5% and 10% DMSO. In general, R5 resins were affected by water storage indicating susceptibility to water degradation, which was not altered by incorporation of low weight percentages of DMSO. However, the storage in water at 37 °C for 30 d may show the maximum level of resin-solvent mixture degradation, curiously, 10% DMSO R5 was not statistically different to 5% DMSO R5 after water storage, albeit a significant decrease occurred for 5% DMSO but not for 10% DMSO R5. This does not imply higher stability of water. However, it may indicate that 10% DMSO incorporation oversaturates polymer structure to the point where additional water sorption/solubility over 30 days was not so relevant on mechanical strength impairment. Nevertheless, longer storage periods might result in further flexural strength impairments considering high water uptake of R5 resins solvated in DMSO.

Water uptake is determined not only by the degree of hydrophilicity of the materials, but it is also dependent on the presence of residual solvents in the polymer structure (Yiu et al., 2006). Since DMSO incorporation significantly affected water sorption/solubility of the tested resins, the fourth null hypothesis was rejected. In general, R2 resins presented significantly lower water sorption and solubility levels compared to R5 resins due to R2's hydrophobic composition and higher crosslink density, which also plays an important role on water sorption/solubility (Ajithkumar et al., 2000). Polymers with lower crosslink density tend to present higher water sorption levels (Ajithkumar et al., 2000). In addition, DMSO is a highly hygroscopic solvent with high hydrogen bonding affinity for water (Kiefer et al., 2011). Therefore, DMSO incorporation of 5% or above (w/w %) may alter the dynamic exchange of water molecules inside the polymer structure expediting water sorption/solubility. The effect of DMSO incorporation on water sorption was more pronounce in R2 than R5 resins. It should be noted that the increase in water sorption/solubility was not caused by reduced monomer conversion, but due to the incorporation of a polar hygroscopic solvent in the resin blend. Since there is a positive correlation of polymer polarity and water sorption (Ito et al., 2005), incorporation of 5% and 10% DMSO increased the overall resin blend polarity contributing to higher sorption/solubility levels. The rationale that the mass variation of R5 resins with high amounts of DMSO in the first 24 h is lower than corresponding R2 resins is because R5 resins suffered high solubility at early stages, unlike R2 resins. Therefore, incorporation of high amounts of DMSO in the hydrophilic resins dramatically increased solubility levels. It is important to note that the relationship between water sorption/solubility and DMSO content in adhesive resins was concentration dependent: 1% or lower DMSO concentrations had no effect on water sorption/solubility irrespective of resin composition.

5. Conclusion

DMSO-monomer ratio and methacrylate monomer composition are crucial for dental adhesive formulations. Increase in degree of conversions after higher DMSO incorporation did not enhance polymer quality even in hydrophobic resin blends containing higher amounts of cross-link monomers. In fact, higher DMSO amounts may impair the mechanical properties and water sorption/solubility levels of methacrylate-based bonding resins regardless of adhesive monomer composition. Incorporation of 5 w/w % or more DMSO compromised the quality of polymer network. Reductions in physio/mechanical properties were comparatively more pronounced in hydrophobic resins, even though DMSO-solvated hydrophobic resins clearly outperformed the corresponding hydrophilic resins. Nevertheless, up to 1 w/w % DMSO incorporation in hydrophobic or hydrophilic experimental bonding resins had no effect on their mechanical/physical properties, and may constitute a threshold to improve the clinical performance of dental adhesives with distinct water affinities.

Acknowledgments

This study was funded by the Grant #296653 from the Academy of Finland to AT-M (PI) and EVO funding #13140 of Turku University Hospital to AT-M (PI).

Conflict of interest

The authors of this manuscript disclose no conflict of interest and certify that they have no proprietary, financial or other personal interest of any nature in any product, finding, and/or company that is presented in this article.

References

Ajithkumar, S., Patel, N.K., Kansara, S.S., 2000. Sorption and diffusion of organic solvents through interpenetrating polymer networks (IPNs) based on polyurethane and unsaturated polyester. Eur. Polym. J. 36, 2387–2393. https://doi.org/10.1016/S0014-3057(00)00025-2.

Bae, J.H., Cho, B.H., Kim, J.S., Kim, M.S., Lee, I.B., Son, H.H., et al., 2005. Adhesive layer properties as a determinant of dentin bond strength. J. Biomed. Mater. Res - Part B Appl. Biomater. 74, 822–828. https://doi.org/10.1002/jbm.b.30320.

Barszczewska-Rybarek, I., Jurczyk, S., 2015. Comparative study of structure-property relationships in polymer networks based on bis-GMA, TEGDMA and various urethane-dimethacrylates. Materials 8, 1230–1248. https://doi.org/10.3390/ma8031230.

Cadenaro, M., Breschi, L., Rueggeberg, F.A., Suchko, M., Grodin, E., Agee, K., et al., 2009. Effects of residual ethanol on the rate and degree of conversion of five experimental resins. Dent. Mater. 25, 621–628. https://doi.org/10.1016/j.dental.2008.11.005.

Dickens, S.H., Stansbury, J.W., Choi, K.M., Floyd, C.J.E., 2003. Photopolymerization kinetics of methacrylate dental resins. Macromolecules 36, 6043–6053. https://doi.org/10.1021/ma021675k.

Fang, M., Liu, R., Xiao, Y., Li, F., Wang, D., Hou, R., et al., 2012. Biomodification to dentin by a natural crosslinker improved the resin-dentin bonds. J. Dent. 40, 458–466. https://doi.org/10.1016/j.jdent.2012.02.008.

Ferracane, J.L., 2006. Hygroscopic and hydrolytic effects in dental polymer networks. Dent. Mater. 22, 211–222. https://doi.org/10.1016/j.dental.2005.05.005.

Floyd, C.J.E., Dickens, S.H., 2006. Network structure of Bis-GMA- and UDMA-based resin systems. Dent. Mater. 22, 1143–1149. https://doi.org/10.1016/j.dental.2005.10.009.

Frassetto, A., Breschi, L., Turco, G., Marchesi, G., Di Lenarda, R., Tay, F.R., et al., 2016. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability - a literature review. Dent. Mater. 32, e41–e53. https://doi.org/10.1016/j.dental.2015.11.007.

Gupta, S.N., Nandi, U.S., 1970. Role of dimethyl sulfoxide as a solvent for vinyl polymerization. J. Polym. Sci. Part A-1 Polym. Chem. 8, 1493–1501. https://doi.org/10.1002/pol.1970.150080614.

Holmes, R.G., Rueggeberg, F.A., Callan, R.S., Caughman, F., Chan, D.C.N., Pashley, D.H., et al., 2007. Effect of solvent type and content on monomer conversion of a model resin system as a thin film. Dent. Mater. 23, 1506–1512. https://doi.org/10.1016/j.dental.2007.01.007.

Ito, S., Hashimoto, M., Wadgaonkar, B., Svizero, N., Carvalho, R.M., Yiu, C., et al., 2005. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 26, 6449–6459. https://doi.org/10.1016/j.biomaterials.2005.04.052.

Jacobsen, T., Söderholm, K.J.M., 1998. Effect of primer solvent, primer agitation, and dentin dryness on shear bond strength to dentin. Am. J. Dent. 11, 225–228.

Kiefer, J., Noack, K., Kirchner, B., 2011. Hydrogen Bonding in Mixtures of Dimethyl Sulfoxide and cosolvents. Curr. Phys. Chem. 1, 340–351. https://doi.org/10.2174/1877947611101040340.

Leitune, V.C.B., Collares, F.M., Trommer, R.M., Andrioli, D.G., Bergmann, C.P., Samuel, S.M.W., 2013. The addition of nanostructured hydroxyapatite to an experimental adhesive resin. J. Dent. 41, 321–327. https://doi.org/10.1016/j.jdent.2013.01.001.

Lemon, M.T., Jones, M.S., Stansbury, J.W., 2007. Hydrogen bonding interactions in methacrylate monomers and polymers. J. Biomed. Mater. Res - Part A 83, 734–746. https://doi.org/10.1002/jbm.a.31448.

Malacarne, J., Carvalho, R.M., de Goes, M.F., Svizero, N., Pashley, D.H., Tay, F.R., et al., 2006. Water sorption/solubility of dental adhesive resins. Dent. Mater. 22, 973–980. https://doi.org/10.1016/j.dental.2005.11.020.

Moraes, R.R. De, Schneider, L.F.J., Correr-Sobrinho, L., Consani, S., Sinhoreti, M.A.C., 2007. Influence of ethanol concentration on softening tests for cross-link density evaluation of dental composites. Mater. Res 10, 79–81. https://doi.org/10.1590/S1516-14392007000100017.

MRDO, Carrilho, Tay, F.R., Pashley, D.H., Tjäderhane, L., Carvalho, R.M., 2005. Mechanical stability of resin-dentin bond components. Dent. Mater. 21, 232–241. https://doi.org/10.1016/j.dental.2004.06.001.

Pashley, D.H., Tay, F.R., Carvalho, R.M., Rueggeberg, F.A., Agee, K.A., Carrilho, M., et al., 2007. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. Am. J. Dent. 20, 7–20.

Pashley, D.H., Tay, F.R., Breschi, L., Tjäderhane, L., Carvalho, R.M., Carrilho, M., et al., 2011. State of the art etch-and-rinse adhesives. Dent. Mater. 27, 1–16. https://doi.org/10.1016/j.dental.2010.10.016.

Pashley, David H., Sano, Hideiko, Ciucchi, Bernard, Masahiro Yoshiyama, R.M.C., 1995. Adhesioon testing of dentin bonding agents: a review. Dent. Mater. 11, 117–125. https://doi.org/10.1016/j.dental.2009.03.015.

Pashley, E.L., Zhang, Y., Lockwood, P.E., Rueggeberg, F.A., Pashley, D.H., 1998. Effects of HEMA on water evaporation from water – HEMA mixtures. Dent. Mater. 5641, 6–10.

Pick, B., Meira, J.B.C., Driemeier, L., Braga, R.R., 2010. A critical view on biaxial and short-beam uniaxial flexural strength tests applied to resin composites using Weibull, fractographic and finite element analyses. Dent. Mater. 26, 83–90. https://doi.org/10.1016/j.dental.2009.09.002.

Rodrigues, S.B., Collares, F.M., Leitune, V.C.B., Schneider, L.F.J., Ogliari, F.A., Petzhold, C.L., et al., 2015. Influence of hydroxyethyl acrylamide addition to dental adhesive resin. Dent. Mater. 31, 1579–1586. https://doi.org/10.1016/j.dental.2015.10.005.

Rueggeberg, F.A., Hashinger, D.T., Fairhurst, C.W., 1990. Calibration of FTIR conversion analysis of contemporary dental resin composites. Dent. Mater. 6, 241–249. https://doi.org/10.1016/S0109-5641(05)80005-3.

Schneider, L.F.J., Moraes, R.R., Cavalcante, L.M., Sinhoreti, M.A.C., Correr-Sobrinho, L., Consani, S., 2008. Cross-link density evaluation through softening tests: effect of ethanol concentration. Dent. Mater. 24, 199–203. https://doi.org/10.1016/j.dental.2007.03.010.

Sideridou, I., Tserki, V., Papanastasiou, G., 2002. Effect of chemical structure on degree of conversion in light-cured dimethacrylate-based dental resins. Biomaterials 23, 1819–1829. https://doi.org/10.1016/S0142-9612(01)00308-8.

Sideridou, I., Tserki, V., Papanastasiou, G., 2003. Study of water sorption, solubility and modulus of elasticity of light-cured dimethacrylate-based dental resins. Biomaterials 24, 655–665. https://doi.org/10.1016/S0142-9612(02)00380-0.

Stape, T.H.S., Tezvergil-Mutluay, A., Mutluay, M.M., Martins, L.R.M., do Prado, R.L., Pizi, E.C.G., et al., 2016. Influence of dimethyl sulfoxide used as a solvent on the physical properties and long-term dentin bonding of hydrophilic resins. J. Mech. Behav. Biomed. Mater. 64, 220–228. https://doi.org/10.1016/j.jmbbm.2016.07.003.

Tay, F.R., Pashley, D.H., 2003. Have dentin adhesives become too hydrophilic? J. Can. Dent. Assoc. 69, 726–731. https://doi.org/10.1016/S0109-5641(03)00110-6.

Tjäderhane, L., Nascimento, F.D., Breschi, L., Mazzoni, A., Tersariol, I.L.S., Geraldeli, S., et al., 2013. Strategies to prevent hydrolytic degradation of the hybrid layer - a review. Dent. Mater. 29, 999–1011. https://doi.org/10.1016/j.dental.2013.07.016.

Van Landuyt, K.L., Snauwaert, J., De Munck, J., Peumans, M., Yoshida, Y., Poitevin, A., et al., 2007. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials 28, 3757–3785. https://doi.org/10.1016/j.biomaterials.2007.04.044.

Ye, Q., Spencer, P., Wang, Y., Misra, A., 2007. Relationship of solvent to the photopolymerization process, properties, structure in model dentin adhesives. J. Biomed. Mater. Res - Part A 80, 342–350. https://doi.org/10.1002/jbm.a.30890.

Yiu, C.K.Y., Pashley, E.L., Hiraishi, N., King, N.M., Goracci, C., Ferrari, M., et al., 2005. Solvent and water retention in dental adhesive blends after evaporation. Biomaterials 26, 6863–6872. https://doi.org/10.1016/j.biomaterials.2005.05.011.

Yiu, C.K.Y., King, N.M., Carrilho, M.R.O., Sauro, S., Rueggeberg, F.A., Prati, C., et al., 2006. Effect of resin hydrophilicity and temperature on water sorption of dental adhesive resins. Biomaterials 27, 1695–1703. https://doi.org/10.1016/j.biomaterials.2005.09.037.